

Title: COMPOSITIONS INVOLVING M-RIP, AND RELATED  
METHODS FOR SCREENING FOR ANTI-HYPERTENSIVE  
AGENTS, AND USES THEREOF

Applicant(s): Michael E. Mendelsohn et al.

Filing Date: 11/17/03

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1A

Human: 1	MSAAKENPCRKFQANIFNKSQCNCFKPREPHLLNDEDLTQAKPIYGGWLLLAPDGTDFD	60
Mouse: 1	MSAAKENPCRKFQANIFNKSQCNCFKPRE HLLNDEDLTQAKPIYGGWLLLAPDGTDFD	60
Human: 61	NPVHRSRKWQRRFFILYEHGLLRYALDEMP TTLPQGTINMNQCTDVVDGEGRTGQKFSLC	120
Mouse: 61	NPVHRSRKWQRRFFILYEHGLLRYALDEMP TTLPQGTINMNQCTDVVDGE RTGQKFSLC	120
Human: 121	ILTPEKEHFIRAETKEIVSGWLEMLMVYPRTNKQNKQKKRKRKEPPTPQEPGPAKVAVTSS	180
Mouse: 121	ILTP+KEHFIRAETKEI+SGWLEMLMVYPRTNKQNKQKKRKRKEPPTPQEPGPAK+AVTSS	180
Human: 181	S--SSSSSSSIPSAEKVPTTKSTLWQEEMRTKDQPDGSSLSPAQSPSQSPPAASSLREP	238
Mouse: 181	S +S SSSSIPSAEKVPTTKSTLWQEEMR KDQPDG+SLSPAQSPSQSPPAA + REP	240
Human: 239	GLESKEEESAMSSDRMDCGRKVRVESGYFSLEKTKQDLKAEQQLPPPLSPSPSTPNHR	298
Mouse: 241	GLESKE+ES +S DR+D GRKVRVESGYFSLEK QDL+AEEQ LPP LSPSPSTP+ R	299
Human: 299	RSQVIEKFEALDIEKAEHMETNA-VGPSPSSDTRQGRSEKRAFPRKRDFNEAPPAPLPD	357
Mouse: 300	RSQVIEKFEALDIEKAEHMETN + +PSSDTRQGRSE+RA PRKRDF +EAP APL D	359
Human: 358	ASASPLSPHRRAKSLDRRSTEPSVTPDLLNFKKGWLTKQYEDGQWKKHWFVLADQSLRYY	417
Mouse: 360	A PLSPHRRAKSLDRRSTE S+TPDLLNFKKGWLTKQYEDGQWKKHWFVLADQSLRYY	417
Human: 418	RDSVAEEAADLDGEIDL SACYDVTEYPVQ RNYGFQIHTKEGEFTLSAMTSGIRRNWIQTI	477
Mouse: 418	RDSVAEEAADLDGEI+LS CYDVTEYPVQ RNYGFQIHTKEGEFTLSAMTSGIRRNWIQTI	477
Human: 478	MKHVHPTTAPDVTSSLPEEKNKSSCSFETCPRPTEKQEAELGEPDPEQKTSRARERRREG	537
Mouse: 478	MKHV P +APDVTSSLPE KNKS+ SFETC R TEKQAE GEPDPEQK SRARERRREG	536
Human: 538	RSKTFDWAEFRPIQQALAQER VGGVGPADTHEPLRPEAESGELERERARRREERRKRFGM	597
Mouse: 537	RSKTFDWAEFRPIQQALAQER VG +D+ +P EAE GELERERARRREE RKRFGM	596
Human: 598	LDATDGP GTEDAALRMEVDRSPGL-PMSDLKTHNVHVEIEQRWHQVETTP LREEKQVPPIA	656
Mouse: 597	LD DGPG ED ALRM++DRSPGL DLKT NVHVEIEQRWHQVETTP LREEKQVPPIA	656
Human: 657	PVHLSSDGGDRLSTHELTSLLEKELEQSQKEASDLLEQNRLLDQLRVALGREQSAREG	716
Mouse: 657	P+HLS ED +RLSTHELTSLLEKELEQSQKEASDLLEQNRLLDQLRVALGREQSAREG	716
Human: 717	YVLQATCERGFAAMEETHQKKIEDLQRQHORELEKLREEKDRLLAEETAATISAIEAMKN	776
Mouse: 717	YVLQATCERGFAAMEETHQKKIEDLQRQHORELEKLREEKDRLLAEETAATISAIEAMKN	776
Human: 777	AHREEMERELEKSQRSQISSVNSDVEALRRQYLEELQSVQRELEV LSEQYSQKCLENAHL	836
Mouse: 777	AHREEMERELEKSQRSQISS+NSD+EALRRQYLEELQSVQRELEV LSEQYSQKCLENAHL	836

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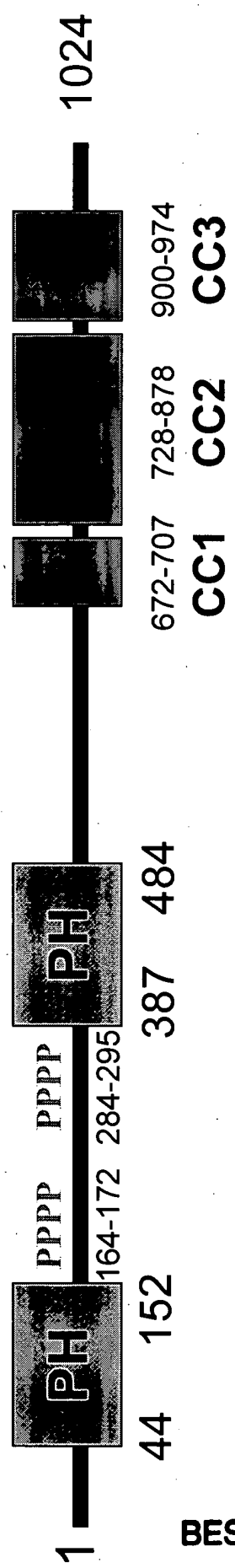
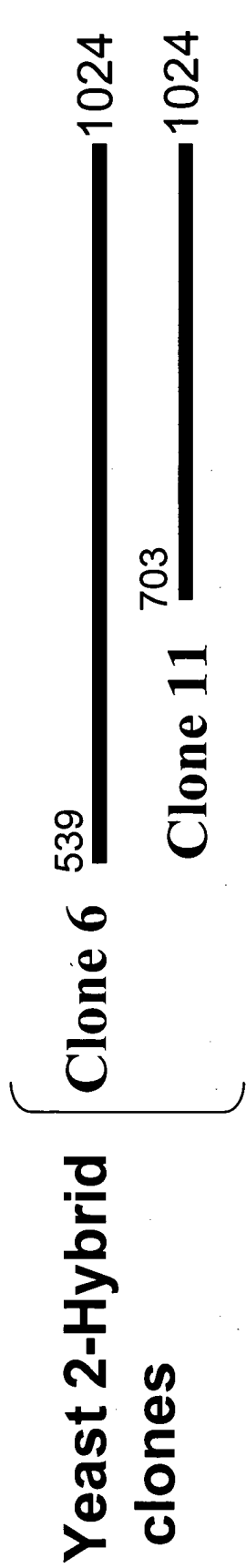
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Mouse: 837 AQALEAERQALRQCQRENQELNAHNQELNNRLAAEITRLRTLTLTGDDGGGESTGLPLTQGK 896

Human: 897 DAYELEVLLRVKESEIQYLKQEISSLKDELQ TALRDKKYASDKYKDIYTELSIAKAKADC 956  
DAYELEVLLRVKESEIQYLKQEISSLKDELQ TALRDKKYASDKYKDIYTELSIAKAKADC  
Mouse: 897 DAYELEVLLRVKESEIQYLKQEISSLKDELQ TALRDKKYASDKYKDIYTELSIAKAKADC 956

Human: 957 DISRLKEQLKAATEALGEKSPDSATVSGYDIMKSKSNPDFLKKDRSCVTRQLRNIRSKSV 1016  
DISRLKEQLKAATEALGEKSP+ TVSGYDIMKSKSNPDFLKKDRSCVTRQLRNIRSKSV  
Mouse: 957 DISRLKEQLKAATEALGEKSPEGTTVSGYDIMKSKSNPDFLKKDRSCVTRQLRNIRSKSV 1016

Human: 1017 IEQVSWDT 1024  
IEQVSWD  
Mouse: 1017 IEQVSWDN 1024

1B



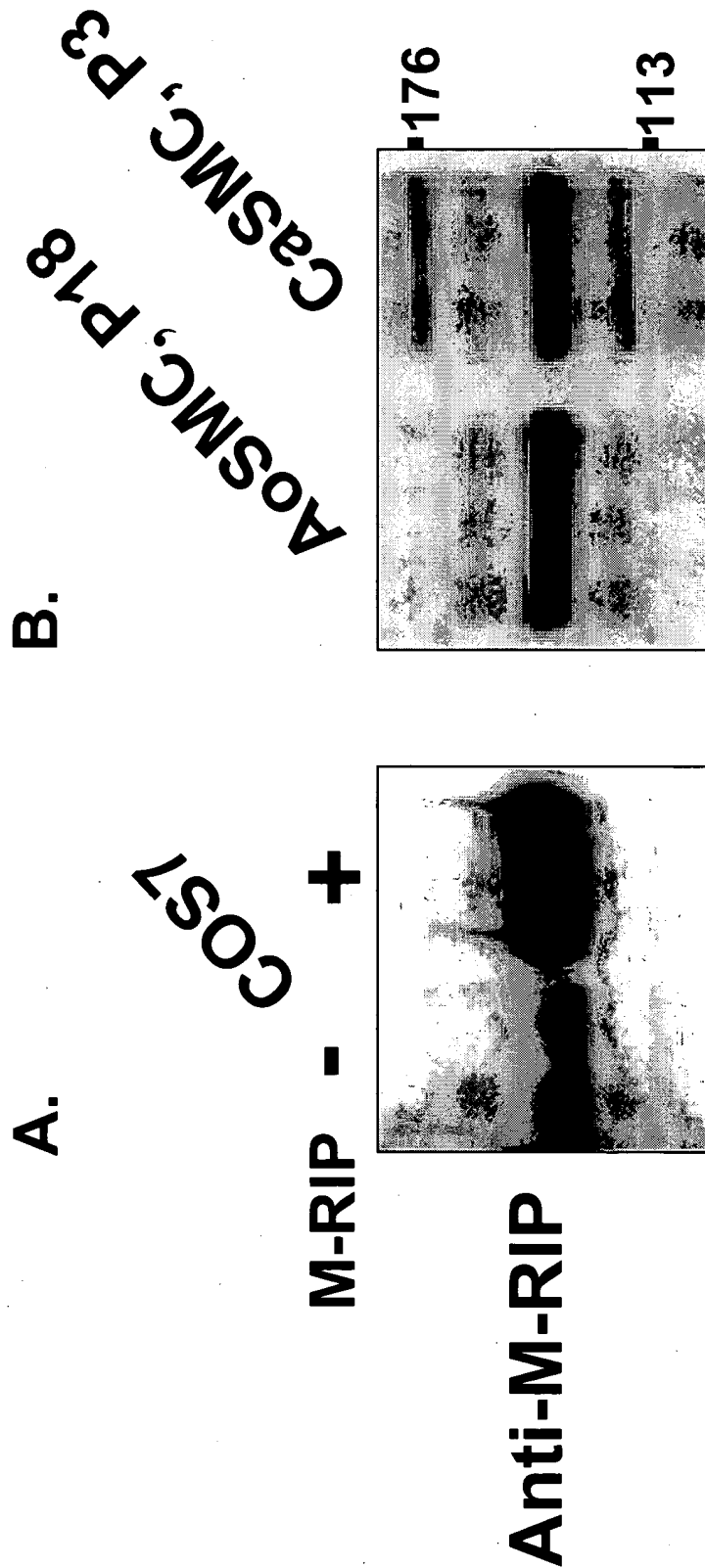
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## FIGURE 1C

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GAGATGCTCATGGTCTATCCCCGGACCAACAAGCAGAATCAGAAGAAGAAACGGAAAGTGGAGCCCCCAC  
ACCACAGGAGCCTGGGCCTGCCAAGGTGGCTGTTACCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCATCC  
CCAGTGCTGAGAAAGTCCCCACCACCAAGTCCACACTCTGGCAGGAAGAAATGAGGACCAAGGACCAGCCA  
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CTTCCAGATACATACAAAGGAGGGCGAGTTTACCCTGTGCGCCATGACATCTGGGATTGCGCGAACTGGA  
TCCAGACCATCATGAAGCACGTGCACCCGACCACTGCCCCGGATGTGACCAGCTCGTTGCCAGAGGAAAAA  
AACAAGAGCAGCTGCTCTTTTGAGACCTGCCCCAGGCCTACTGAGAAGCAAGAGGCAGAGCTGGGGGAGCC  
GGACCCTGAGCAGAAGACGAGCCGCGCACGGGAGCGGAGGCGAGAGGGCCGCTCCAAGACCTTTGACTGGG  
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GAGCCCCCTGCGCCCTGAGGCGGAGTCTGGGGAGCTGGAGCGGGAGCGTGCACGGAGGCGGGAGGAGCGCCG  
CAAGCGCTTCGGGATGCTCGACGCCACAGACGGGCCAGGCACTGAGGATGCAGCCCTGCGCATGGAGGTGG  
ACCGGAGCCCAGGGCTGCCTATGAGCGACCTCAAAACGCATAACGTCCACGTGGAGATTGAGCAGCGGTGG  
CATCAGGTGGAGACCACACCTCTCCGGGAAGAGAAGCAGGTGCCCATCGCCCCGTCCACCTGTCTTCTGA  
AGATGGGGGTGACCGGCTCTCCACACACGAGCTGACCTCTCTGCTCGAGAAGGAGCTGGAGCAGAGCCAGA  
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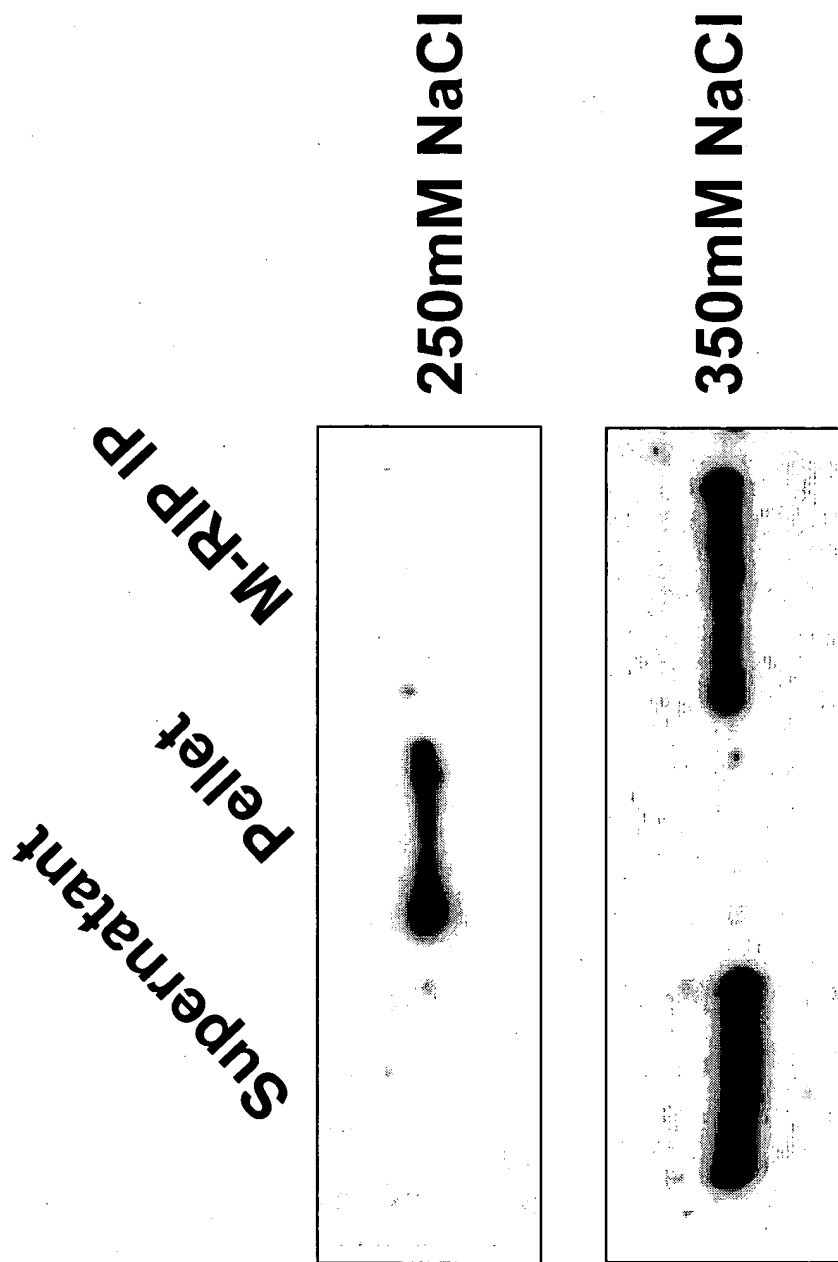
**Figure 2**



# Anti-M-RIP

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2C



2D

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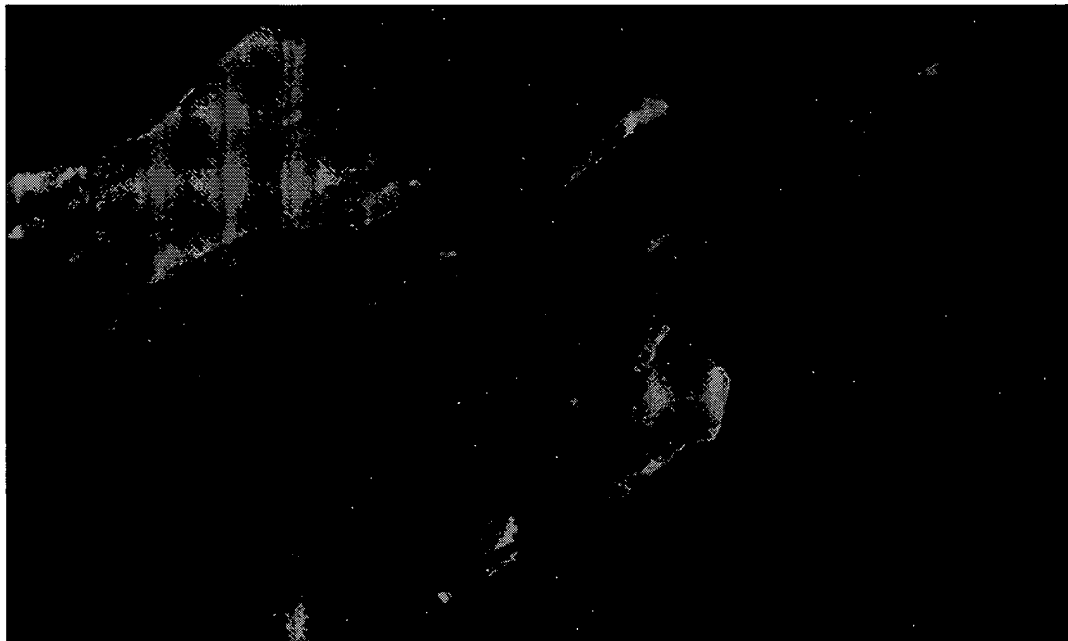
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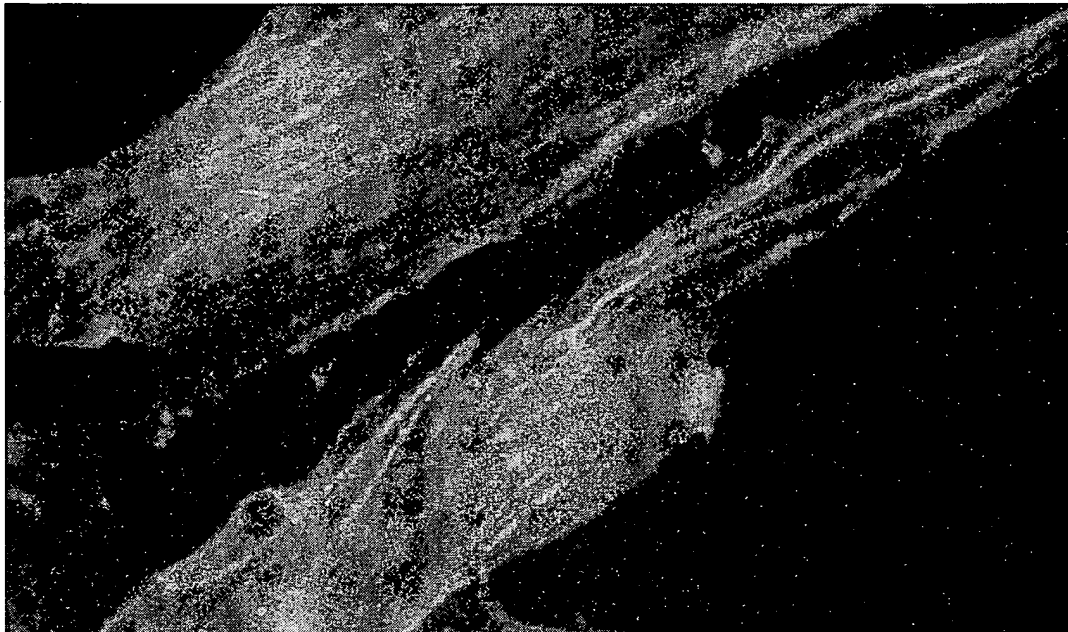
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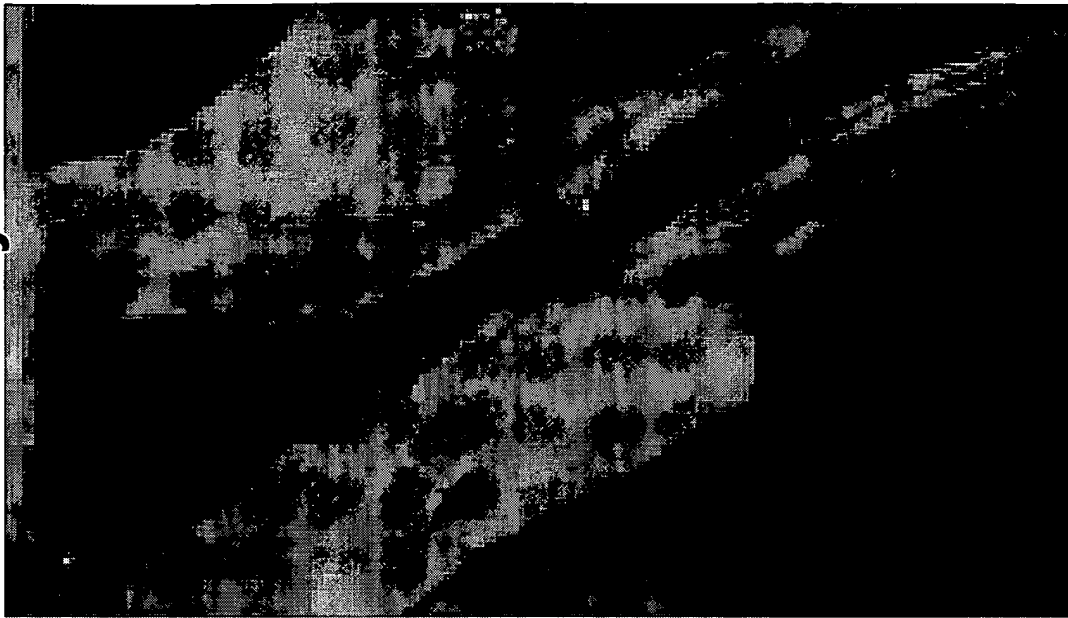
Anti-M-RIP



Phalloidin



Overlay



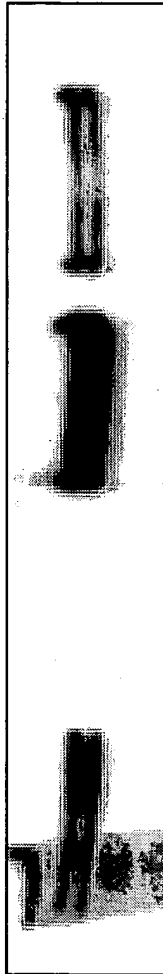
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3A

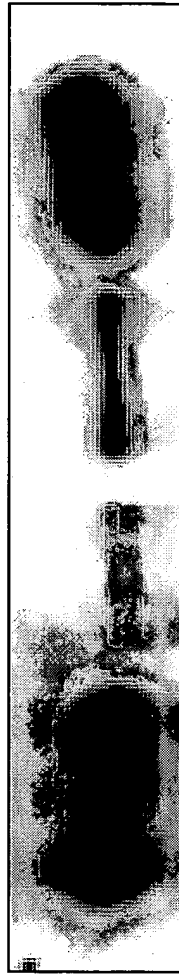
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5% Input  
Nonimmune IgG  
MBS IP  
M-RIP IP

Anti-MBS



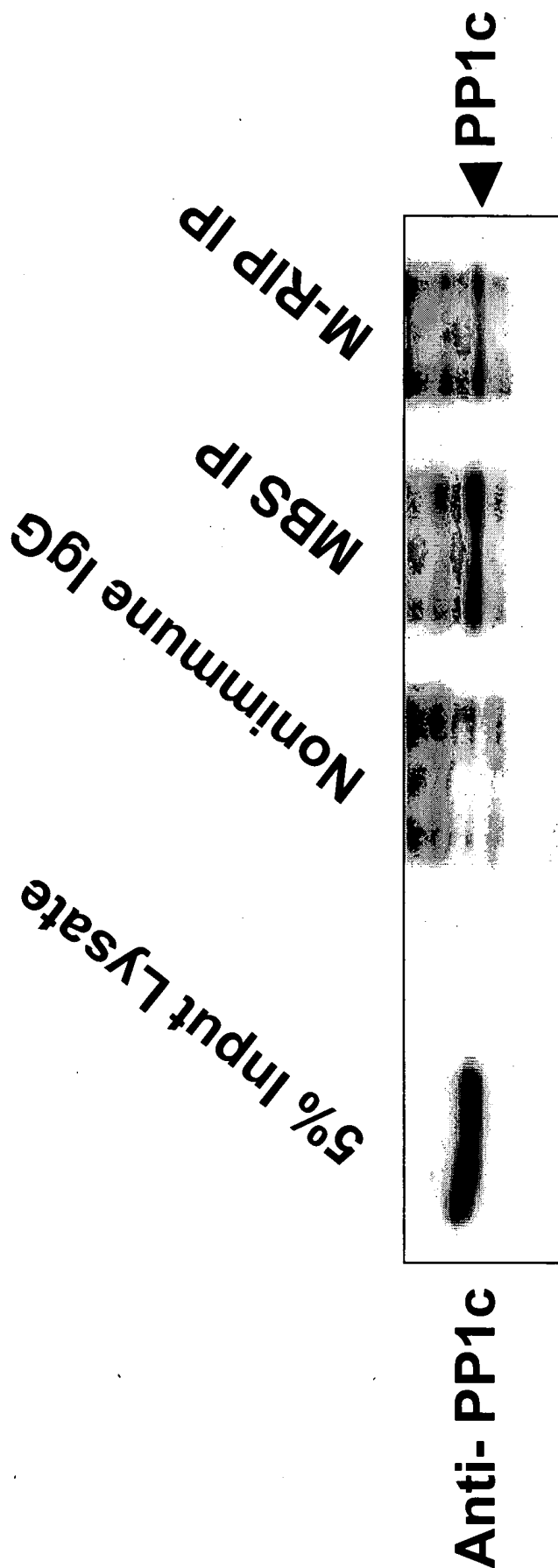
Anti-M-RIP





3B

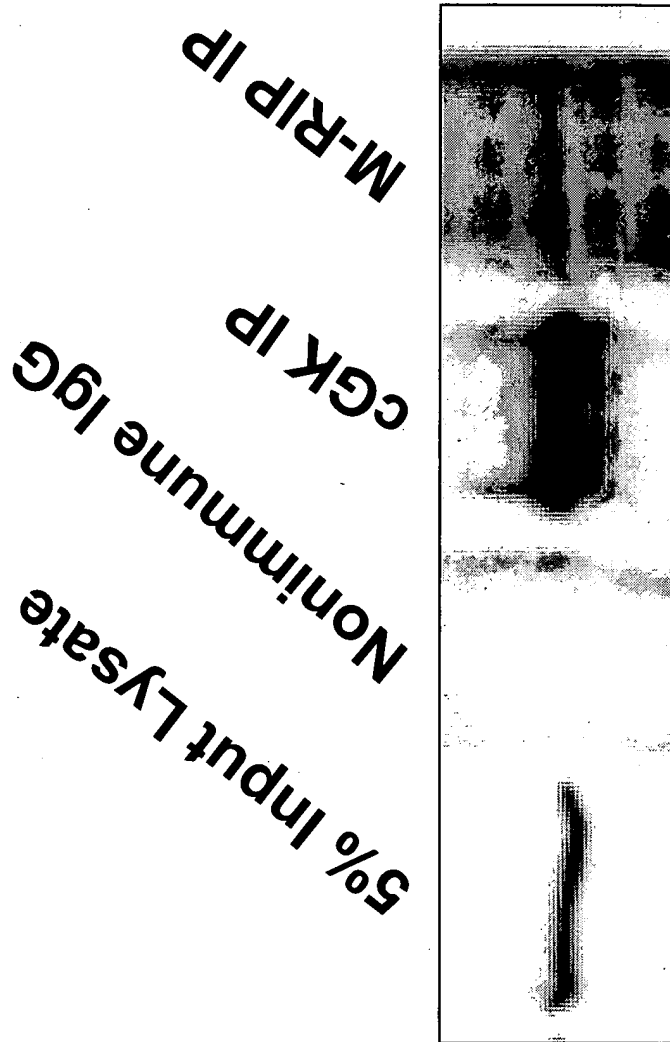
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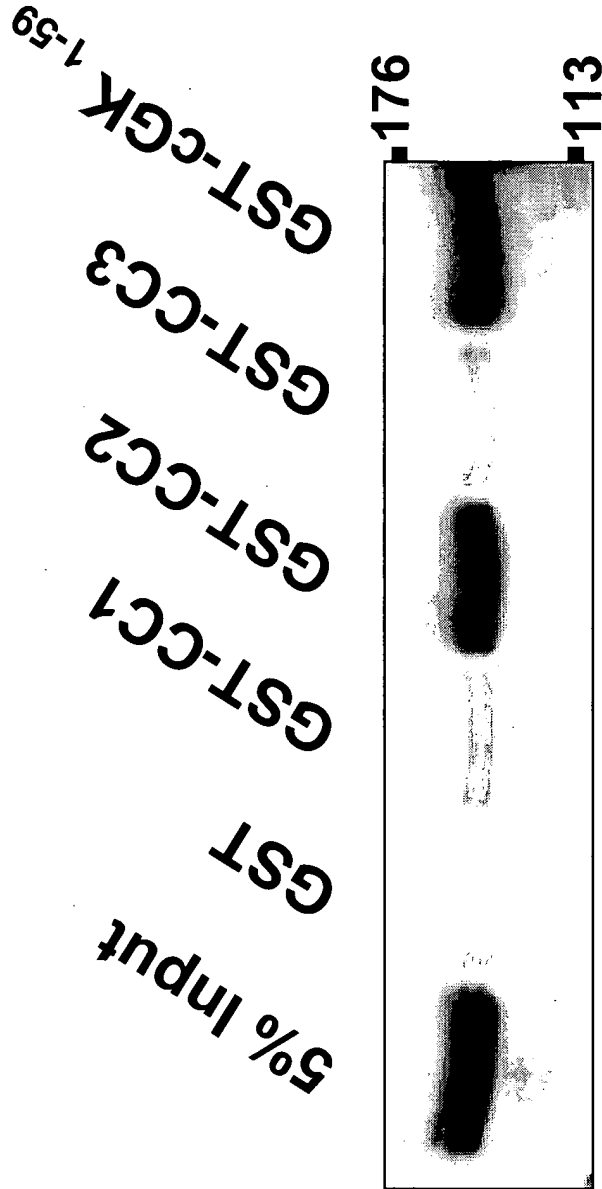
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## Anti- cGK



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# Anti-MBS



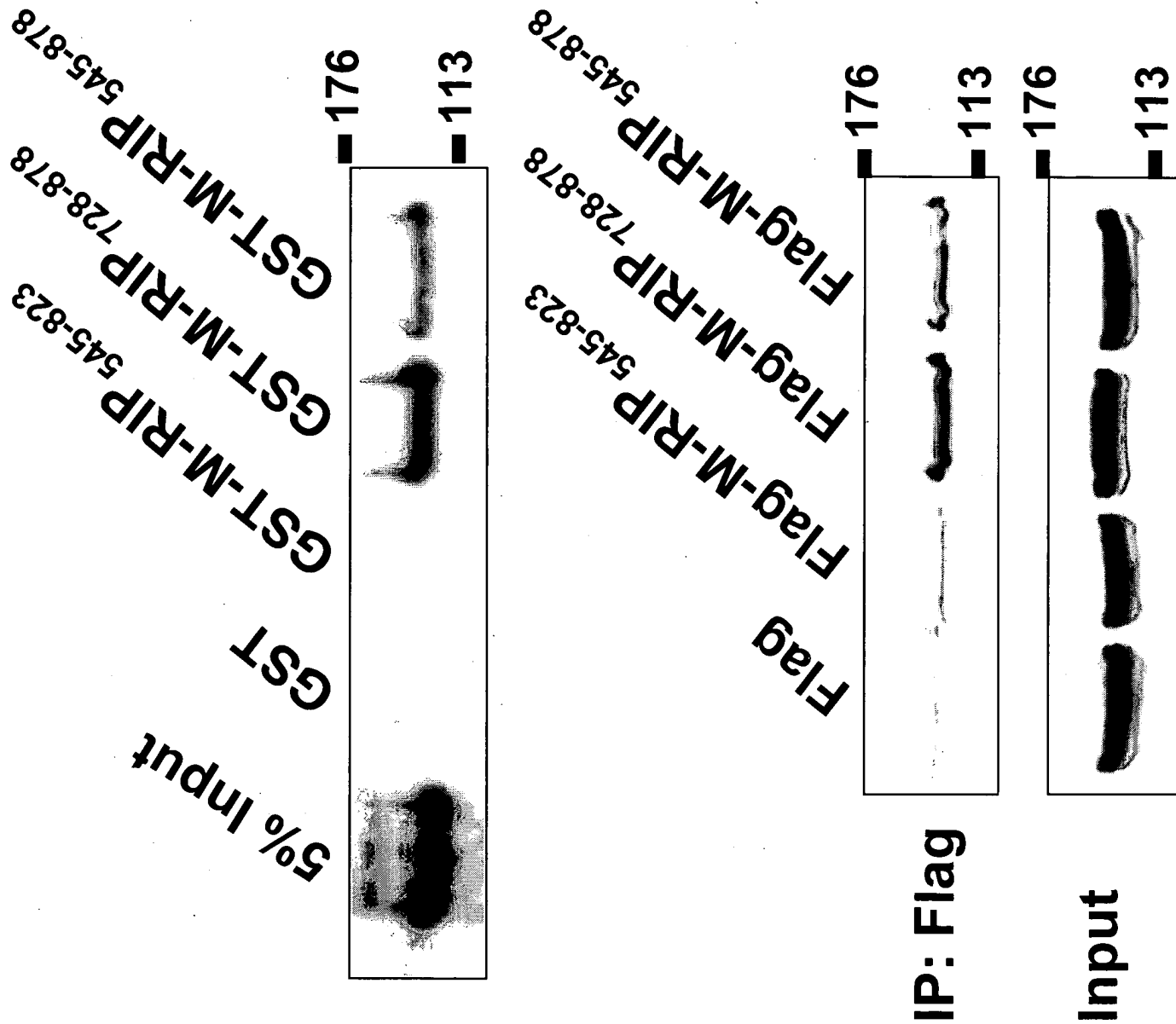
4B

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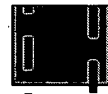
## Anti-MBS

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Amino Acids    MBS Binding

CC1    CC2    CC3



672-707 (CC1)

-



728-878 (CC2)

+



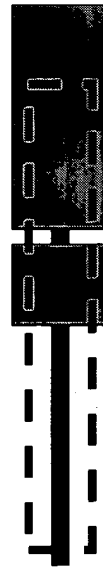
900-974 (CC3)

-



545-823

-

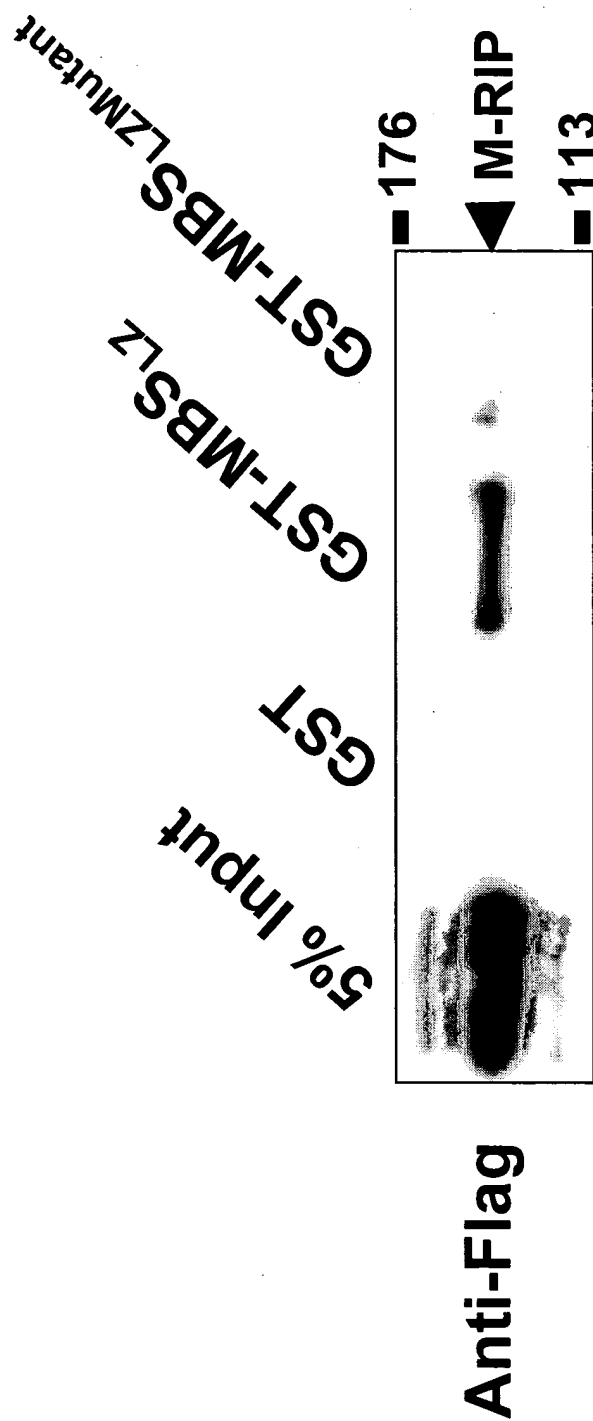


545-878

+

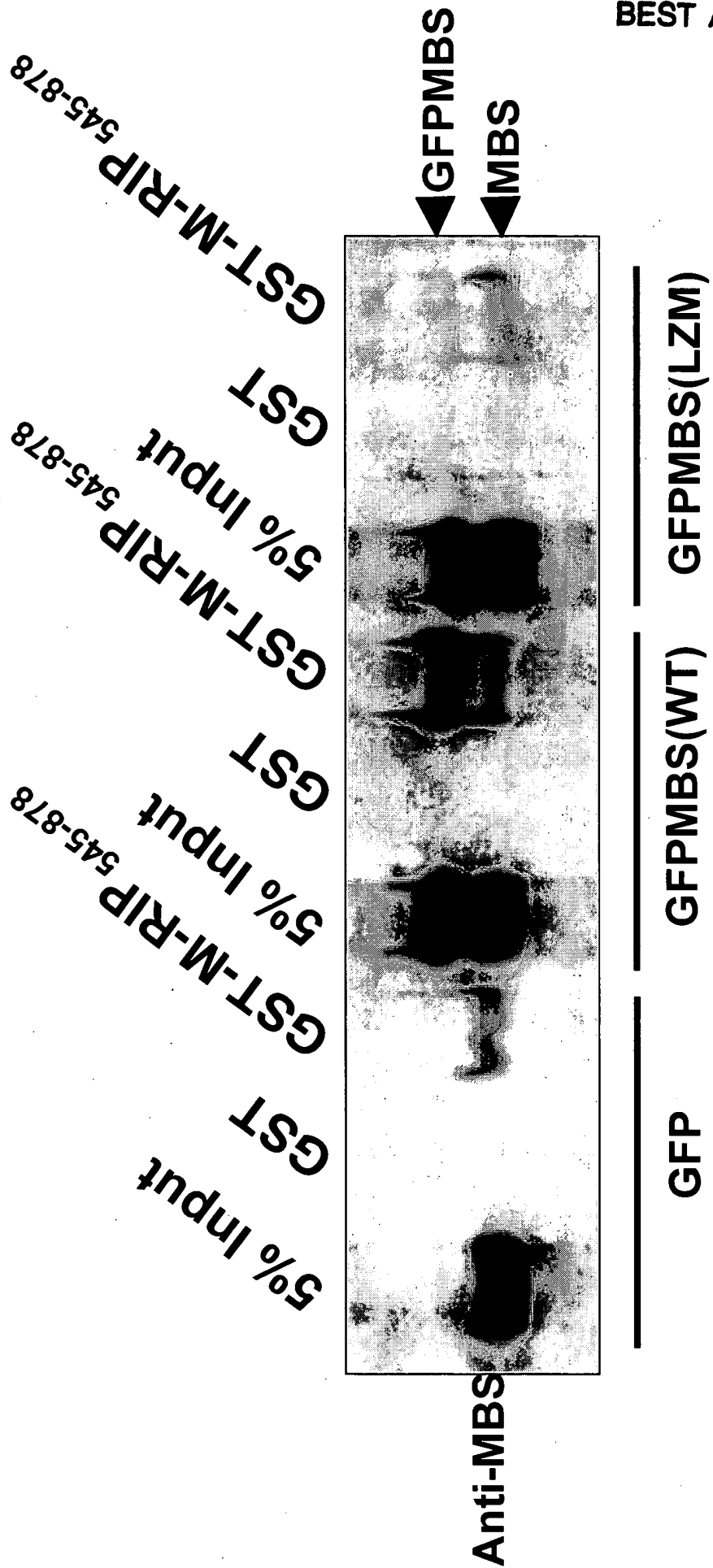
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4E

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4F

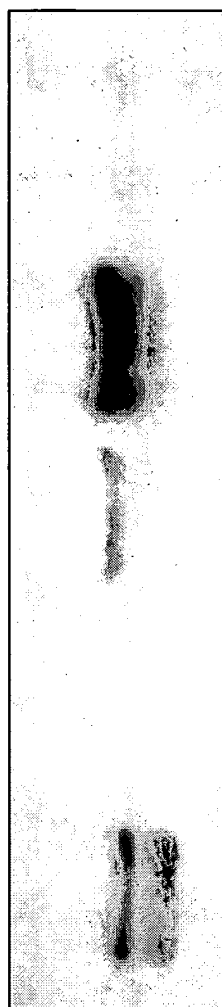
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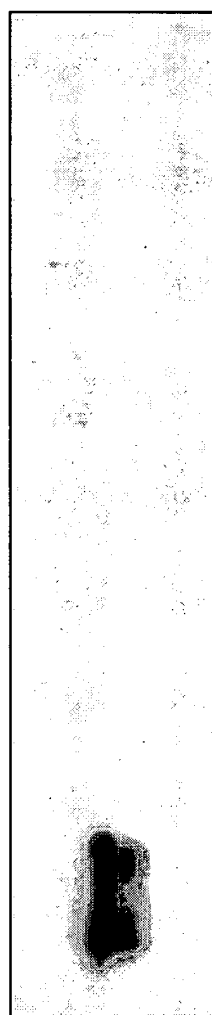
Input  
Control  
HisCC1  
HisCC2  
HisCC3

↓ GST-MBSLZ

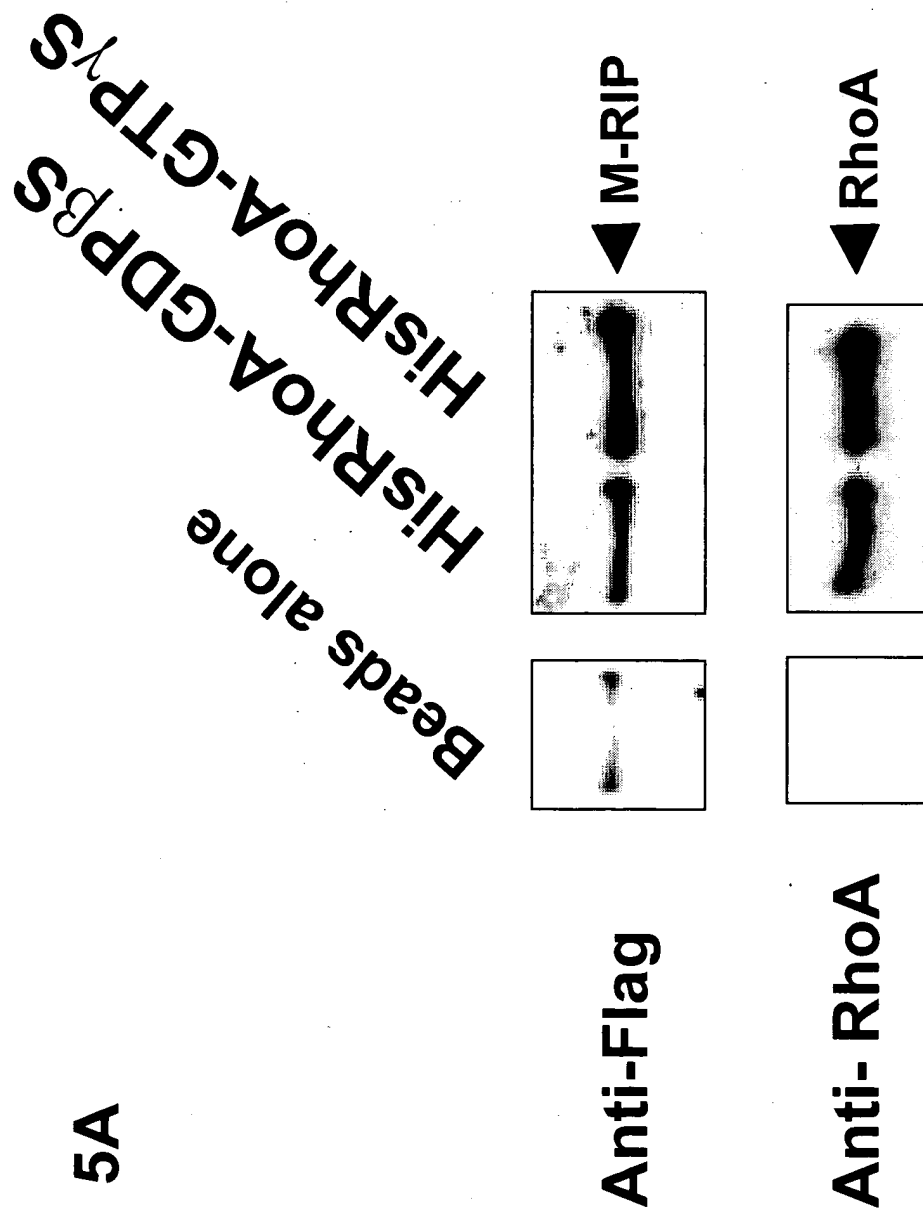
↓ GST-MBSLZmutant



Anti-GST







5B

GDPBS GTPys GDPBS GTPys GDPBS GTPys GDPBS GTPys

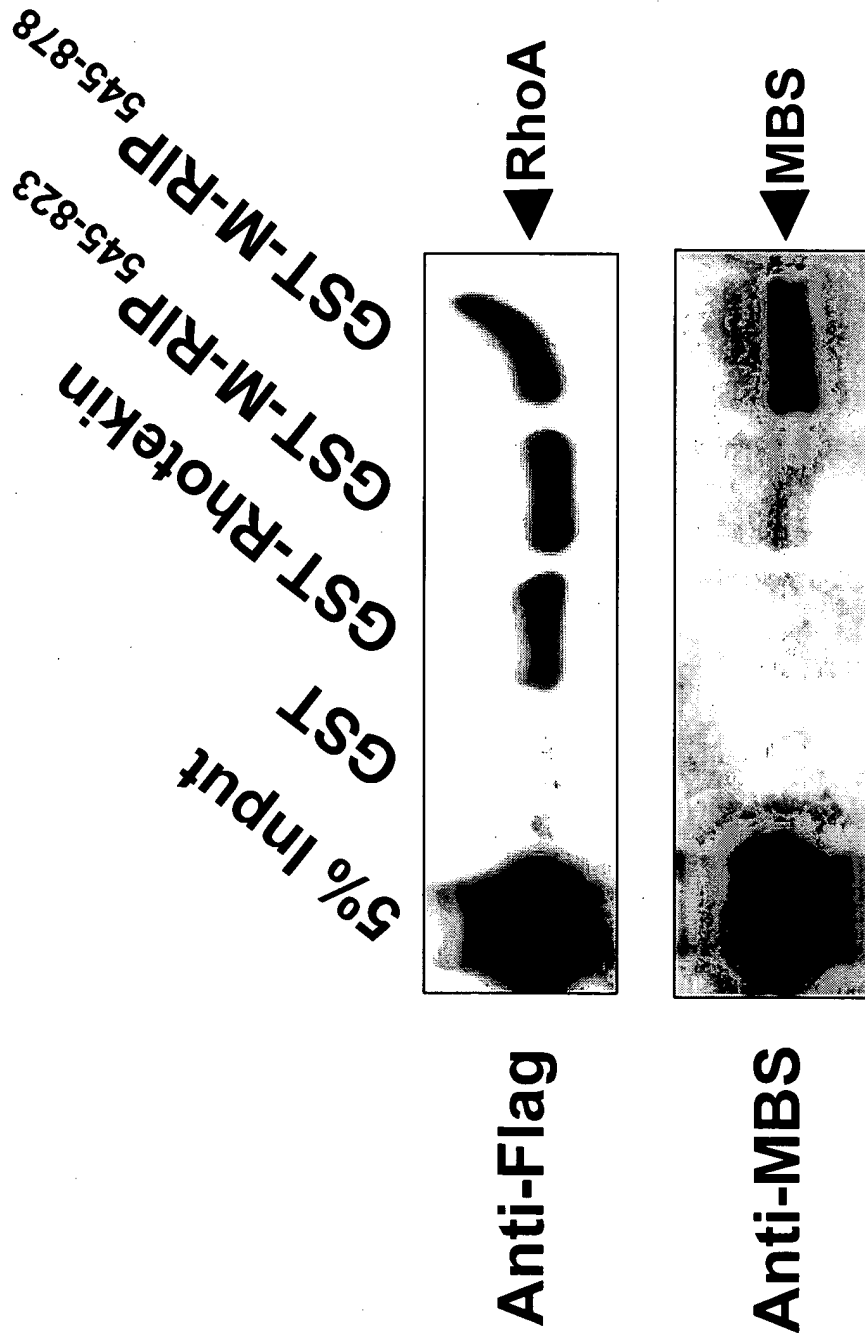
HisRhoA+:

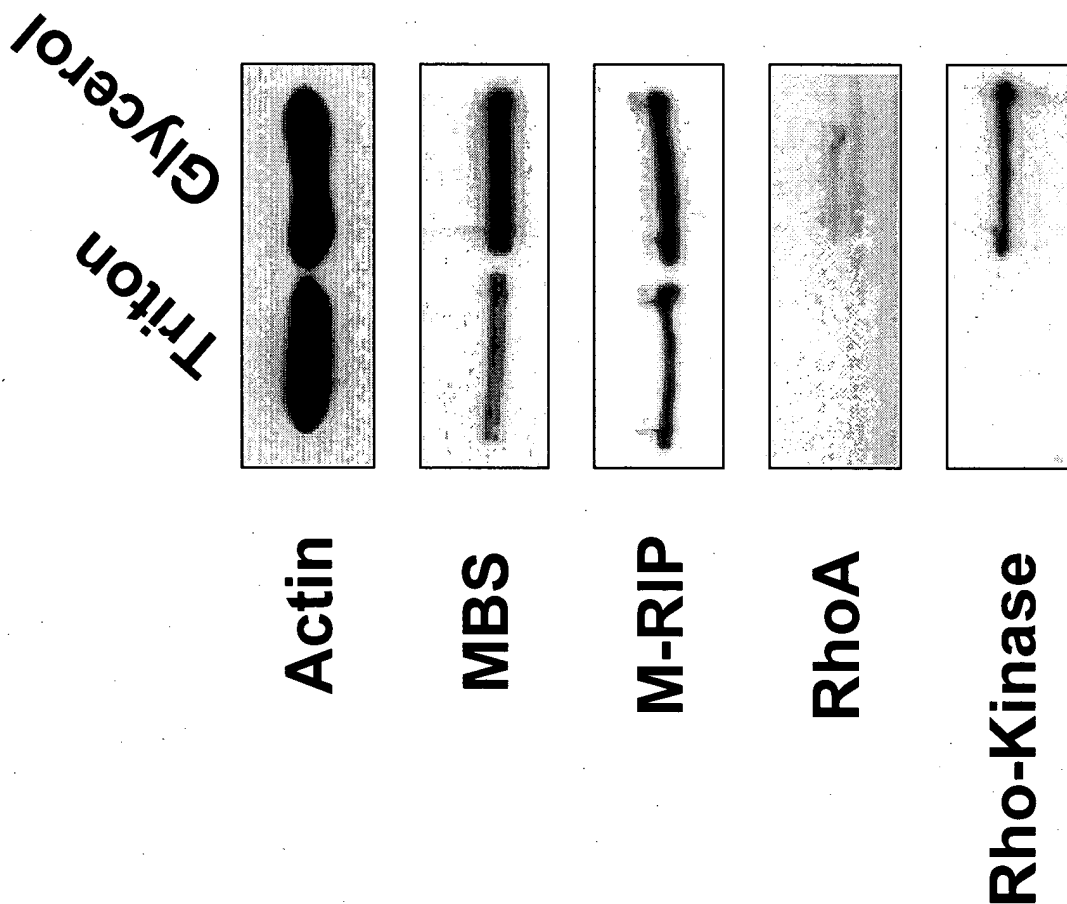


Anti-RhoA

GST GST- Rhotekin<sub>RBD</sub> M-RIP<sub>545-823</sub> GST- M-RIP<sub>545-878</sub>

5C





6A

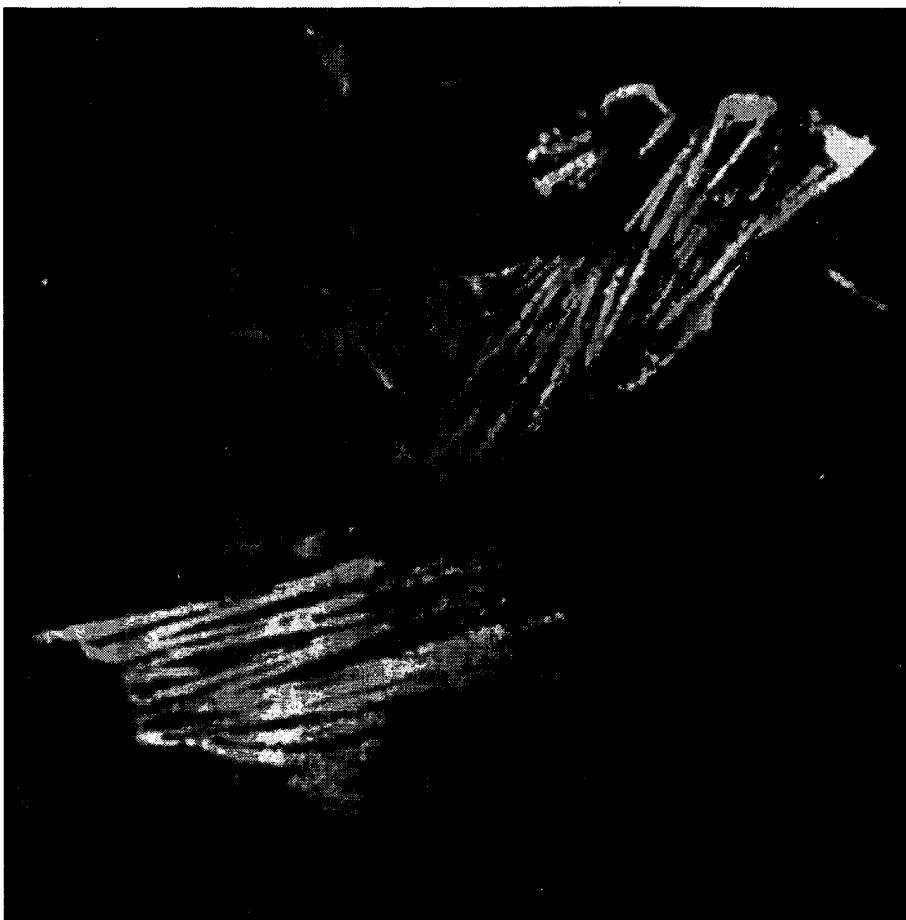
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6B, top

Phalloidin



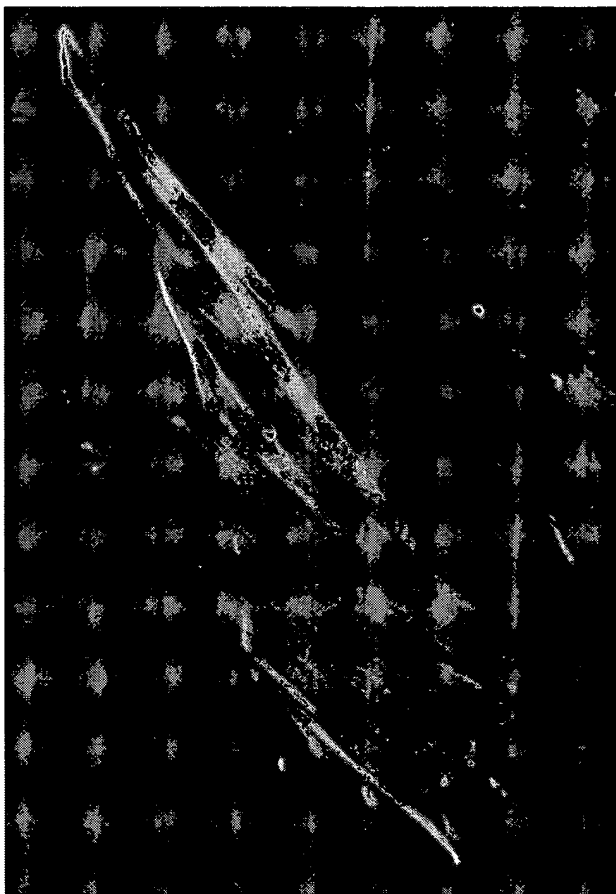
M-RIP



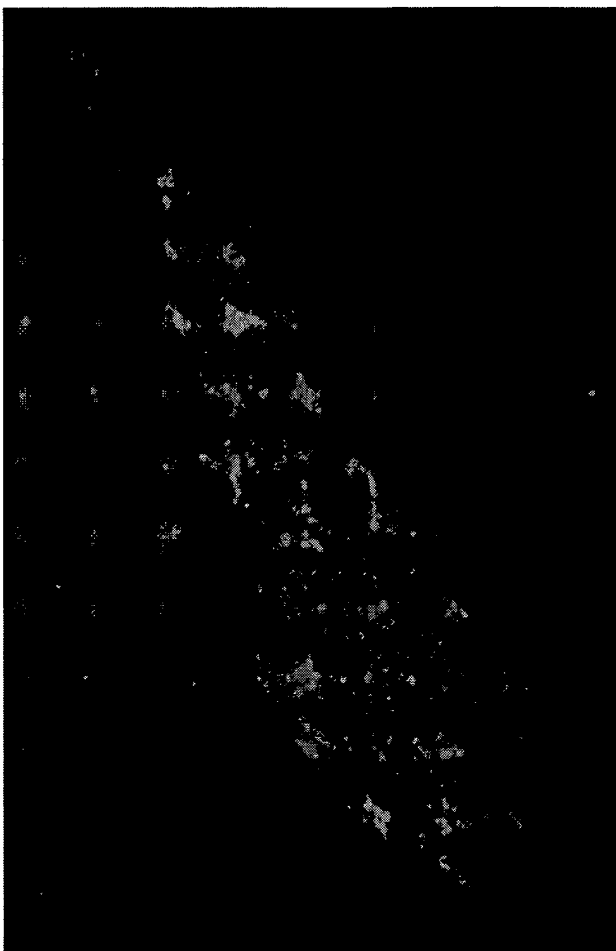
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**6B, middle**

**Phalloidin**



**MBS**



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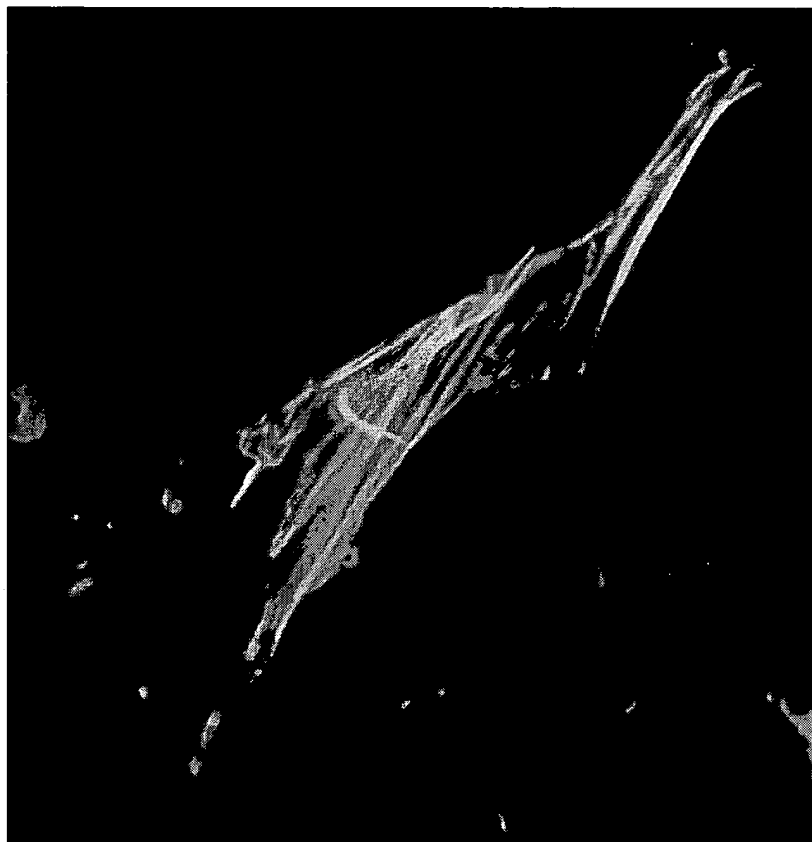
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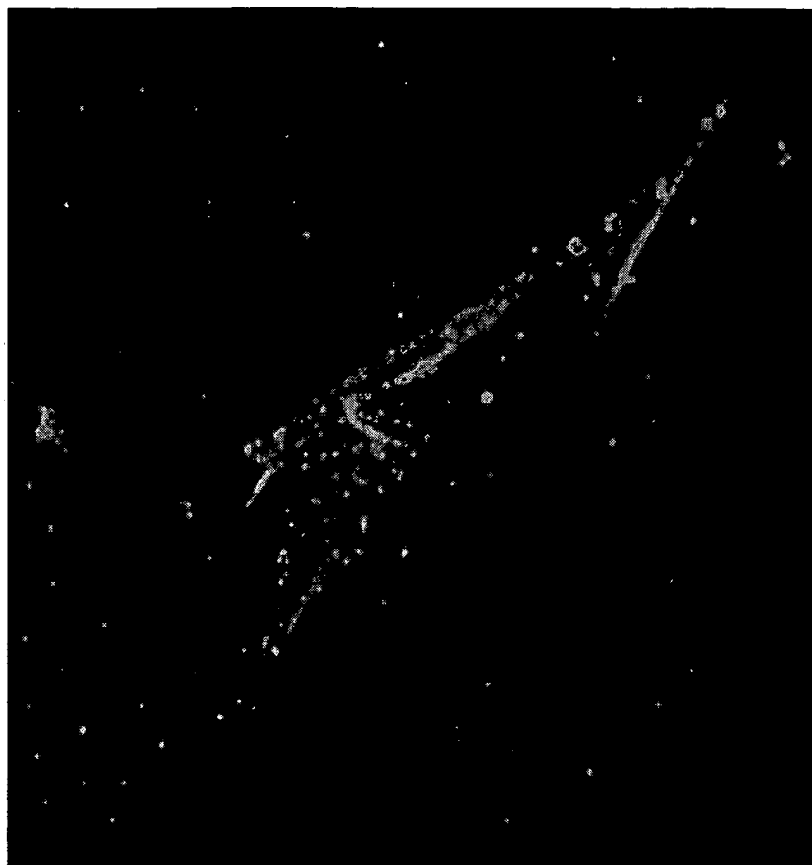
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6B, bottom

Phalloidin

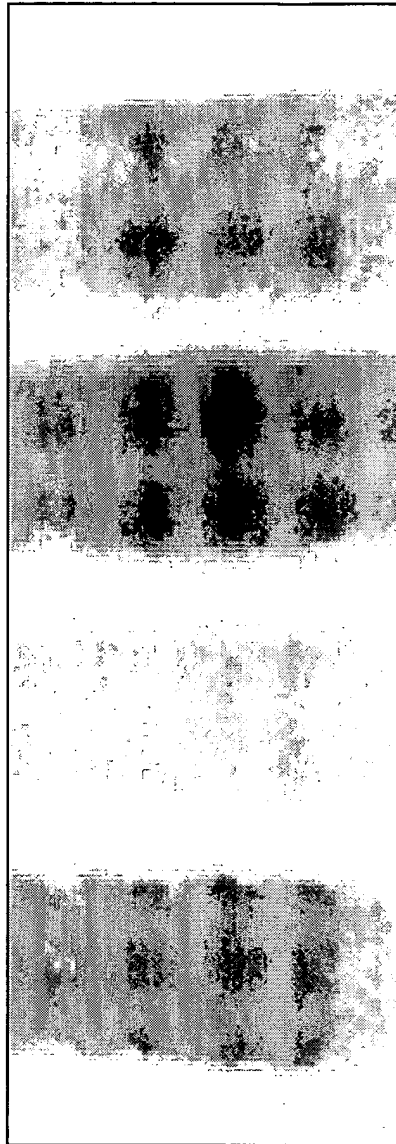


RhoA



6C

IP:NI IP:M-RIP



Y27632 1μM

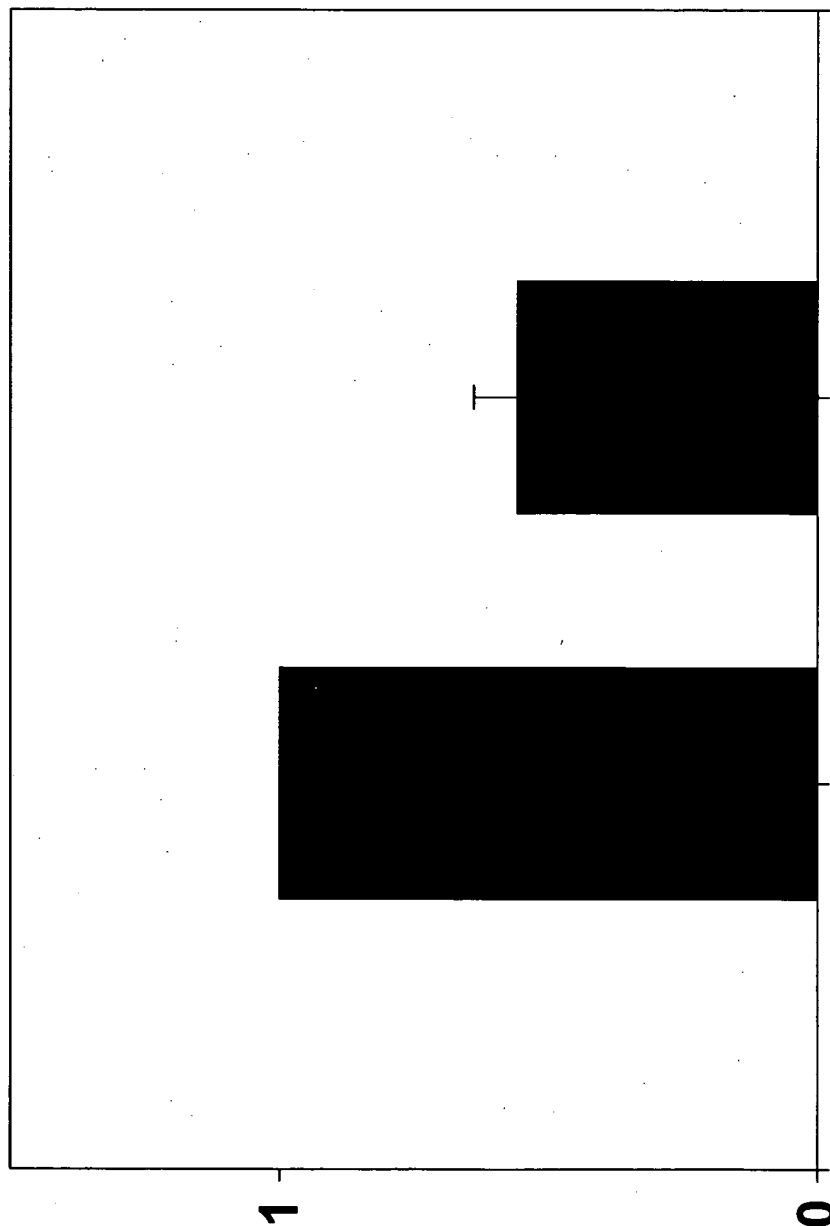
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6D

Arbitrary Densitometric Units



Y27632

The diagram illustrates the RhoA signaling pathway. RhoA (a circle) is activated by M-RIP (a rectangle) and inhibits MBS (a rectangle). MBS is a component of the LZ (a rectangle). The M-RIP is composed of PH (a rectangle) and MBS-BD (a rectangle). The MBS-BD is composed of Rho-BD (545-823) and MBS (823-878).